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Male Health

The preoperative serum cystatin-C as an independent prognostic factor for survival in upper tract urothelial carcinoma

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Cystatin-C (Cys-C) has been reported as a valuable prognostic biomarker in various malignancies. However, its effect on upper tract urothelial carcinoma (UTUC) patients has not been investigated before. Thus, to explore the impact of Cys-C on survival outcomes in patients undergoing radical nephroureterectomy (RNU), a total of 538 patients with UTUC who underwent RNU between 2005 and 2014 in our center (West China Hospital, Chengdu, China) were included in this study. Kaplan–Meier method and Cox regression analyses were performed to assess the relationship between Cys-C and survival outcomes using SPSS version 22.0. The cutoff value of Cys-C was set as 1.4 mg l^{-1} using the receiver operating characteristic (ROC) curves and Youden index. The mean age of patients included was 66.1 ± 11.1 years, and the median follow-up duration was 38 (interquartile range: 19–56) months. Overall, 162 (30.1%) patients had elevated Cys-C, and they were much older and had worse renal function than those with Cys-C <1.4 mg l^{-1} (both $P < 0.001$). Meanwhile, Kaplan–Meier analysis revealed that the group with elevated Cys-C had worse cancer-specific survival (CSS, $P = 0.001$), disease recurrence-free survival (RFS, $P = 0.003$), and overall survival (OS, $P < 0.001$). Multivariable Cox analysis suggested that the elevated Cys-C was identified as an independent prognostic predictor of CSS (hazard ratio [HR]: 1.997, 95% confidential interval [CI]: 1.331–2.996), RFS (HR: 1.429, 95% CI: 1.009–2.023), and OS (HR: 1.989, 95% CI: 1.366–2.896). In conclusion, our result revealed that the elevated preoperative serum Cys-C was significantly associated with worse outcomes in UTUC patients undergoing RNU.

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INTRODUCTION

Upper tract urothelial carcinoma (UTUC) is a relatively rare malignancy that accounts for only 5%–10% of all urothelial carcinomas.[1] At the moment, many clinical decisions making for UTUC are derived from evidence that based on bladder cancer cohorts, because the upper and lower urinary tract carcinomas share many characteristics. However, previous studies also found that there were many differences in practical, anatomical, biological, and molecular features which benefited risk stratifying and treating strategy making between them.[2–5] The radical nephroureterectomy (RNU) with bladder-cuff resection is still the standard treatment for nonmetastatic UTUC to date.[1] Although the diagnosis and surgical techniques have improved, the survival outcomes have not significantly changed during last few decades.[6]

The current risk stratification of UTUC is mainly based on postoperative data, including pathological tumor (pT) stage, tumor grade, lymphovascular invasion (LVI), lymph node metastasis, and concomitant variant histology (CVH).[1] As the management measures for different risk groups are different according to guidelines, thus it is important for clinicians to find some preoperative predictors to help make management scheme.

Recently, some preoperative parameters such as tumor location, tumor size, hydronephrosis, neutrophil–lymphocyte ratio, and C-reactive protein have been found to be associated with the prognosis of UTUC patients.[7–9] Previous studies have also demonstrated that abnormal serum level of cystatin C (Cys-C) could serve as a diagnostic and prognostic indicator for myeloma, breast cancer, colon cancer, and non-Hodgkin B-cell lymphoma as well as renal cell carcinoma.[10–14] However, the potential role of serum Cys-C in UTUC has never been investigated before. Cys-C has been proposed as an endogenous marker of the glomerular filtration rate (GFR) with higher sensitivity than serum creatinine.[15–17] Moreover, preoperative and postoperative renal function has been well established as an independent prognostic factor in UTUC patients after RNU.[18–21] Cys-C is also an inhibitor of cysteine proteases such as cathepsins (B, D, H, L, and S) and plays a role in the regulation of cell proliferation, differentiation, and migration.[22] Thus, the aim

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of this study was to retrospectively assess the prognostic impact of serum Cys-C on UTUC patients undergoing RNU.

PATIENTS AND METHODS

Patients

A total of 577 patients with UTUC who underwent surgical treatment in West China Hospital (Chengdu, China) between 2005 and 2014 were reviewed, but 31 patients were excluded from the study due to missing data. Furthermore, patients with the previous cystectomy for invasive bladder cancer (n = 3), patients who underwent RNU plus radical cystectomy (n = 3), and those with concomitant nonurothelial carcinoma (n = 2) were also excluded; no patient received neoadjuvant chemotherapy before surgery. The study was approved by the Ethics Committee of West China Hospital, and the methods were carried out in accordance with the approved guidelines. For this retrospective study, the formal consent is not required.

Clinical and pathological data evaluation

All RNU specimens were separately evaluated by two specific pathologists (Huan Xu and Yong Jiang) according to standard procedures. The 2002 American Joint Committee of Cancer TNM classification and the WHO International Society of Urological Pathology consensus classification were used to evaluate the tumor stage and grade, respectively. LVI was defined as the presence of tumor cells within an endothelium-lined space without underlying muscular walls.[23] A positive surgical margin was defined as the presence of the tumor at inked areas of soft tissue on the RNU specimen.[24] Lymph node status was categorized as negative (pN0), unknown (pNx), or positive (pN+).[25] Tumor location was categorized as the renal pelvis, ureter, or involvement of both.[26] Multifocality means two or more tumor sites were found in images or pathological analysis. CVH was defined as the presence of both urothelial carcinoma and variant histological differentiation in the RNU specimens.[27] Preoperative serum Cys-C values were acquired from the routine blood tests within 30 days before surgery from Hospital Information System (HIS) database. Other variables including age, gender, tumor architecture, surgical approach, perioperative blood transfusion, hydronephrosis, tumor size, and adjuvant therapy were also collected.

Follow-up regimen

The follow-up schedule was made according to the guideline.[1] Simply, patients were followed every 3 months for the first year after surgery, semiannually for the 2nd and 3rd year, and annually thereafter, or as clinically indicated. Regular tests included urinary cytology and excretory urography of the contralateral upper urinary tract and routine checkups that included history, physical examination, blood laboratory tests, and chest radiography. If clinically indicated, selective bone scan and chest/abdomen computed tomography (CT)/magnetic resonance imaging (MRI) were elevated.[1]

Disease recurrence was defined as local recurrence in the operating field, lymph node spread, and/or distant metastasis that had not been found in the preoperative examinations. Specifically, the tumor found in the urinary bladder or contralateral upper urinary tract after surgery was not regarded as tumor relapse.

Lymph node dissection was not routinely performed. As the indications for the adjuvant therapy (bladder instillation, systematic chemotherapy, and radiotherapy) are still not clear in European Association of Urology (EAU) guideline for UTUC, so we would recommend a single dose of adjuvant bladder chemotherapy (pirarubicin, mitomycin, or epirubicin) to prevent bladder recurrence after RNU.[1] The systematic chemotherapy (cisplatin-based or noncisplatin-based chemotherapy) or radiotherapy was recommended if 1) the surgical margin or lymph nodes were positive and 2) clinically indicated local or distant recurrence or metastasis during follow-up. But for those with comorbidities and impaired renal function, systematic chemotherapy was recommended with caution as chemotherapy-related toxicity could reduce their survival outcomes.[1]

Statistical analyses

Continuous variables were analyzed using Student's t-test, and categorical variables were evaluated using the Chi-squared test. Logistic regression was performed to assess the relationship between Cys-C and clinicopathological features. The cutoff value of Cys-C was determined using the receiver operating characteristic (ROC) curve and Youden index (Youden index = sensitivity + specifcity − 1), which were commonly used to select the cutoff points for the markers in clinical trials.[28] The value of Cys-C at the point where Youden index was maximum was set as the cutoff value. The Kaplan–Meier method was used to calculate survival outcomes including overall survival (OS), cancer-specific survival (CSS), and disease recurrence-free survival (RFS) in two groups, and the log-rank test was used to assess their differences. Univariable and multivariable Cox proportional hazards regression models were performed to evaluate the relationship between variables and OS, CSS, and RFS. Hazard ratios (HRs) with their 95% confidential intervals (CIs) were used to assess the strength of the individual variables. All reported P values were two-sided with statistical significance set at $P < 0.05$. Statistical analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 538 patients with a mean age of 66.1 (standard deviation [s.d.]: 11.1) years were included in the present study, and the median follow-up duration was 38 (interquartile range: 19–56) months. Among participants, 265 patients (49.3%) had the tumor in the renal pelvis, 180 (33.5%) had tumor only in the ureter, and 93 (17.3%) had tumors involved in both sites. In addition, 182 patients (33.8%) were diagnosed with pTis/Ta/T1, 102 (19.0%) with pT2, 178 (33.1%) with pT3, and 76 (14.1%) with pT4. Positive lymph nodes were found in 48 (8.9%) patients. A total of 103 patients underwent lymph node dissection, and 48 of them were proved to have lymph node metastasis. At the time of analysis, 154 participants died from UTUC, and 180 patients died from all causes as well as 217 patients developed UTUC recurrence. The cutoff value of Cys-C was 1.4 mg l^{-1}. Finally, 162 (30.1%) patients were included in the elevated Cys-C group (Cys-C ≥1.4 mg l^{-1}). Compared with the low Cys-C group, the patients in the elevated Cys-C group were much older and had worse renal function and higher serum creatinine (all $P < 0.001$) (**Table 1**).

Logistic regression found that the age and serum creatinine were positively associated with Cys-C (relative risk [RR]: 1.070, 95% CI: 1.041–1.100 and RR: 1.017, 95% CI: 1.017–1.053, respectively), while estimated glomerular fltration rate (eGFR) was negatively correlated to Cys-C (RR: 0.961, 95% CI: 0.934–0.988) (**Table 2**). In addition, ROC curve analysis found that Cys-C (area under the curve [AUC] = 0.593, $P < 0.001$) was much significant compared with eGFR (AUC = 0.567, $P = 0.012$), but creatinine was not a significant indicator (AUC = 0.542, $P = 0.109$). Kaplan–Meier analysis showed that the group with elevated Cys-C had worse CSS ($P = 0.001$), RFS ($P = 0.003$), and OS ($P < 0.001$) compared with the group with low Cys-C level (**Figure 1**). The overall estimated 5-year CSS, RFS, and OS were 45.3% ± 6.7%, 36.3% ± 5.5%, and 42.3% ± 5.1%, respectively, in the patients with

Table 1: Baseline characteristics of 538 patients with upper tract urothelial carcinoma included in the present study

Variable	*Total (n=538)*	*Cys-C ≥1.4 mg l^{-1} (n=162)*	*Cys-C <1.4 mg l^{-1} (n=376)*	*P*
Age (year), mean±s.d.	66.1±11.1	71.2±9.0	63.8±11.1	<0.001
Gender (male/female), *n*	306/232	93/69	213/163	0.871
LVI (positive/negative), *n*	79/459	33/129	46/330	0.017
CVH (with/without), *n*	112/426	34/128	78/298	0.949
Size (>3 cm/≤3cm), *n*	356/182	116/46	240/136	0.080
Margin status (positive/negative), *n*	42/496	17/145	25/351	0.127
Multifocality (with/without), *n*	93/445	25/137	68/308	0.455
Tumor side (left/right), *n*	270/268	88/74	182/194	0.208
Tumor grade (high/no)	392/146	129/33	263/113	0.020
Tumor site				0.693
Pelvicalyceal, *n* (%)	265 (49.3)	76 (46.9)	189 (50.3)	
Ureteric, *n* (%)	180 (33.5)	55 (34.0)	125 (33.2)	
Both, *n* (%)	93 (17.3)	31 (19.1)	62 (16.5)	
Tumor stage				
pTa/is/1, *n* (%)	182 (33.8)	43 (26.5)	139 (37.0)	0.063
pT2, *n* (%)	102 (19.0)	36 (22.2)	66 (17.6)	
pT3, *n* (%)	178 (33.1)	54 (33.3)	124 (33.0)	
pT4, *n* (%)	76 (14.1)	29 (17.9)	47 (12.5)	
Lymph node status				0.566
pN0, *n* (%)	55 (10.2)	20 (12.3)	35 (9.3)	
pNx, *n* (%)	435 (80.9)	128 (79.0)	307 (81.6)	
pN+, *n* (%)	48 (8.9)	14 (8.6)	34 (9.0)	
Tumor architecture (sessile/papillary), *n*	363/175	119/43	244/132	0.052
eGFR (ml min^{-1} per 1.73 m^2), mean±s.d.	66.2±21.8	48.5±17.1	73.8±19.0	<0.001
Serum creatinine (μmol l^{-1})	104.8±52.6	139.6±80.8	89.8±20.5	<0.001
Surgical approach (laparoscopy/open), *n*	223/315	65/97	158/218	0.682
Perioperative blood transfusion (yes/no), *n*	67/471	24/138	43/333	0.276
Hydronephrosis (yes/no), *n*	333/205	106/56	227/149	0.268
Bladder cancer				0.604
Without, *n* (%)	457 (84.9)	134 (82.7)	323 (85.9)	
With history, *n* (%)	22 (4.1)	7 (4.3)	15 (4.0)	
Concomitant, *n* (%)	59 (11.0)	21 (13.0)	38 (10.1)	
Adjuvant therapy (yes/no), *n*	229/309	54/108	175/201	0.004
Overall mortality, *n* (%)	180 (33.5)	71 (43.8)	109 (29.0)	0.001
Cancer-specific mortality, *n* (%)	154 (28.6)	59 (36.4)	95 (25.3)	0.010
Disease recurrence, *n* (%)	217 (40.3)	77 (47.5)	140 (37.2)	0.028
Lymph node dissection, *n* (%)	103 (19.1)	34 (21.0)	69 (18.4)	0.476

LVI: lymph node invasion; s.d.: standard deviation; CVH: concomitant variant histology; Cys-C: cystatin-C; eGFR: estimated glomerular filtration rate

elevated Cys-C and 66.7% ± 3.2%, 51.9% ± 3.6%, and 62.5% ± 3.3%, respectively, in their counterparts.

In univariable analysis, the results showed that the elevated Cys-C was associated with worse CSS (HR: 1.708, 95% CI: 1.234–2.365; **Table 3**), RFS (HR: 1.513, 95% CI: 1.145–1.999; **Table 4**), and OS (HR: 1.791, 95% CI: 1.327–2.416; **Table 5**). Furthermore, the advanced tumor stage, high tumor grade, positive lymph node and margin status, the presence of LVI, with CVH, size ≥3 cm, sessile architecture, and perioperative transfusion all contributed to worse CSS, RFS, and OS (**Table 3–5**). While, eGFR was only related to RFS ($P = 0.018$; **Table 4**).

In multivariable analysis, the elevated Cys-C was also identified as an independent prognostic factor for CSS (HR: 1.997, 95% CI: 1.331–2.996; **Table 3**), RFS (HR: 1.429, 95% CI: 1.009–2.023; **Table 4**), and OS (HR: 1.989, 95% CI: 1.366–2.896; **Table 5**). The results also suggested that tumor pT stage, lymph node invasion, and tumor size were independent predictors of OS, CSS, and RFS (**Table 3–5**). The high-grade disease was only related to worse CSS (HR: 2.376, 95% CI: 1.316–4.289; **Table 3**) and OS (HR: 1.914, 95% CI: 1.147–3.195; **Table 5**). Interestingly, eGFR and adjuvant therapy both were found to be related to CSS (HR: 1.014, 95% CI: 1.004–1.023 and HR: 0.656, 95% CI: 0.468–0.921, respectively; **Table 3**) and OS (HR: 1.010, 95% CI: 1.001–1.019 and HR: 0.681, 95% CI: 0.498–0.930, respectively; **Table 5**) in multivariable model.

DISCUSSION

This is the first study to evaluate the relationship between preoperative serum Cys-C level and the survival outcomes of UTUC. The results showed that the elevated Cys-C contributed to shorter CSS, RFS, and OS in UTUC patients after RNU treatment. Serum Cys-C was demonstrated as an important biomarker of renal function and even more sensitive for estimating eGFR than serum creatinine.[17] The elevated Cys-C was also found to be related to worse renal function in our study. In addition, the results confirmed that the elevated Cys-C was as an independent prognostic predictor of survival outcomes in UTUC patients in the multivariable analysis. Evidence has proved that renal insufficiency significantly increased the mortality after cancer treatments.[29] The eGFR in our study was calculated using

Table 2: Logistic regression analysis to evaluate the relationship between Cys-C and clinicopathological features in patients with upper tract urothelial carcinoma

Variable	*Univariable analysis*			*Multivariable analysis*		
	RR	*95% CI*	*P*	*RR*	*95% CI*	*P*
Age (per 1 year)	1.078	1.055–1.101	<0.001	1.070	1. 041–1.100	<0.001
Tumor grade (high *vs* low)	1.680	1.080–2.611	0.021	0.971	0.511–1.845	0.927
Margin status (positive *vs* negative)	1.646	0.863–3.140	0.130	-	-	-
LVI (positive *vs* negative)	1.835	1.123–2.999	0.015	1.725	0. 887–3.354	0.108
CVH (with *vs* without)	1.015	0.645–1.596	0.949	-	-	-
Tumor size (>3 cm *vs* ≤3 cm)	1.429	0.957–2.134	0.081	1.641	0.959–2.808	0.071
Tumor architecture (sessile *vs* papillary)	1.497	0.996–2.251	0.053	1.311	0.735–2.339	0.360
Hydronephrosis (yes *vs* no)	1.242	0.846–1.824	0.268	-	-	-
Surgical approach (laparoscopic *vs* open)	0.925	0.635–1.345	0.682	-	-	-
Perioperative blood transfusion (yes *vs* no)	1.347	0.787–2.305	0.277	-	-	-
Tumor stage (≥pT3 *vs* ≤pT2)	1.260	0.871–1.822	0.220	-	-	-
Lymph node status						
pNx *vs* pN0	0.730	0.406–1.312	0.292	-	-	-
pN+ *vs* pN0	0.721	0.314–1.653	0.439	-	-	-
eGFR (per 1 ml min^{-1} per 1.73 m^2)	0.910	0.894–0.927	<0.001	0.961	0.934–0.988	0.005
Serum creatinine (per 1 μmol l^{-1})	1.058	1.047–1.070	<0.001	1.035	1.017–1.053	<0.001

RR: relative risk; CI: confidential interval; LVI: lymph node invasion; CVH: concomitant variant histology; Cys-C: cystatin-C; eGFR: estimated glomerular filtration rate. -: not included in the analysis

Table 3: Univariable and multivariable Cox regression analyses of urinary tract urothelial carcinoma with regard to cancer-specific survival

Variable	*Univariable analysis*			*Multivariable analysis*		
	HR	*95% CI*	*P*	*HR*	*95% CI*	*P*
Age (per 1 year)	0.998	0.984–1.013	0.821	-	-	-
Sex (male *vs* female)	0.780	0.568–1.070	0.123	-	-	-
Tumor site			0.836			
Pelvicalyceal		Reference				
Ureteric	0.898	0.626–1.288	0.558	-	-	-
Both	0.987	0.638–1.527	0.945	-	-	-
Hydronephrosis (yes *vs* no)	1.150	0.827–1.599	0.407	-	-	-
Tumor grade (high *vs* low)	4.176	2.450–7.116	<0.001	2.376	1.316–4.289	0.004
Margin status (positive *vs* negative)	2.035	1.228–3.372	0.006	0.827	0.479–1.427	0.496
LVI (positive *vs* negative)	3.024	2.118–4.317	<0.001	1.301	0.862–1.962	0.210
CVH (with *vs* without)	2.472	1.768–3.455	<0.001	1.463	1.021–2.095	0.038
Tumor size (>3 cm *vs* ≤3 cm)	1.972	1.366–2.846	<0.001	1.490	1.007–2.204	0.046
Tumor architecture (sessile *vs* papillary)	3.450	2.214–5.375	<0.001	1.349	0.808–2.252	0.252
Surgical approach (laparoscopic *vs* open)	0.676	0.479–0.953	0.025	0.877	0.609–1.263	0.479
Perioperative blood transfusion (yes *vs* no)	2.351	1.606–3.441	<0.001	1.610	1.061–2.443	0.025
Tumor stage			<0.001			<0.001
pTa/is/1		Reference			Reference	
pT2 *vs* pTa/is/1	1.301	0.683–2.478	0.423	1.033	0.525–2.033	0.924
pT3 *vs* pTa/is/1	4.047	2.501–6.551	<0.001	2.616	1.500–4.560	0.001
pT4 *vs* pTa/is/1	9.363	5.636–15.553	<0.001	3.917	1.998–7.680	<0.001
Lymph node status			<0.001			0.008
pN0		Reference			Reference	
pNx *vs* pN0	1.686	0.880–3.231	0.115	2.453	1.260–4.773	0.008
pN+ *vs* pN0	6.042	2.974–12.279	<0.001	3.258	1.539–6.899	0.002
eGFR (per 1 ml min^{-1} per 1.73 m^2)	0.996	0.989–1.004	0.343	1.014	1.004–1.023	0.004
Cys-C (≥1.4 mg l^{-1} *vs* <1.4 mg l^{-1})	1.708	1.234–2.365	0.001	1.997	1.331–2.996	0.001
Adjuvant therapy (yes *vs* no)	0.814	0.591–1.121	0.208	0.656	0.468–0.921	0.015

HR: hazard ratio; CI: confidential interval; LVI: lymph node invasion; CVH: concomitant variant histology; Cys-C: cystatin-C; eGFR: estimated glomerular filtration rate. -: not included in the analysis

serum creatinine levels, and our results also found that the eGFR was a significant prognostic factor in UTUC patients, like the findings in other tumors.[19–21] Thus, the prognostic impact of Cys-C in UTUC may be partly attributed to its correlation with renal function. In addition, adjuvant therapy was proved to be related to CSS and OS in multivariable analysis, which was partially consistent with the results

Table 4: Univariable and multivariable Cox regression analyses of urinary tract urothelial carcinoma with regard to disease recurrence-free survival

Variable	*Univariable analysis*			*Multivariable analysis*		
	HR	*95% CI*	*P*	*HR*	*95% CI*	*P*
Age (per 1 year)	0.998	0.987–1.011	0.803	-	-	-
Sex (male *vs* female)	0.811	0.621–1.059	0.124	-	-	-
Tumor site			0.687			
Pelvicalyceal		Reference				
Ureteric	0.874	0.645–1.186	0.388	-	-	-
Both	0.964	0.667–1.392	0.844	-	-	-
Hydronephrosis (yes *vs* no)	1.343	1.011–1.785	0.042	1.057	0.781–1.431	0.720
Tumor grade (high *vs* low)	2.593	1.789–3.758	<0.001	1.456	0.957–2.215	0.079
Margin status (positive *vs* negative)	1.789	1.150–2.784	0.010	0.772	0.479–1.245	0.289
LVI (positive *vs* negative)	2.562	1.873–3.504	<0.001	1.064	0.733–1.543	0.745
CVH (with *vs* without)	2.166	1.620–2.896	<0.001	1.317	0.966–1.797	0.082
Tumor size (>3 cm *vs* ≤3 cm)	1.844	1.362–2.497	<0.001	1.464	1.063–2.016	0.020
Tumor architecture (sessile *vs* papillary)	2.694	1.917–3.787	<0.001	1.366	0.912–2.046	0.130
Surgical approach (laparoscopic *vs* open)	0.867	0.656–1.146	0.318	1.091	0.810–1.470	0.567
Perioperative blood transfusion (yes *vs* no)	1.746	1.225–2.489	0.002	1.410	0.959–2.074	0.081
Tumor stage			<0.001			<0.001
pTa/is/1		Reference			Reference	
pT2 *vs* pTa/is/1	1.408	0.866–2.290	0.167	1.096	0.657–1.830	0.726
pT3 *vs* pTa/is/1	3.397	2.317–4.979	<0.001	2.373	1.527–3.687	<0.001
pT4 *vs* pTa/is/1	7.819	5.159–11.850	<0.001	3.988	2.290–6.945	<0.001
Lymph node status			<0.001			0.005
pN0		Reference			Reference	
pNx *vs* pN0	1.599	0.954–2.678	0.075	2.084	1.226–3.542	0.007
pN+ *vs* pN0	5.203	2.902–9.328	<0.001	2.795	1.502–5.200	0.001
eGFR (per 1 ml min^{-1} per 1.73 m^2)	0.992	0.986–0.999	0.018	1.002	0.993–1.010	0.714
Cys-C (≥1.4 mg l^{-1} *vs* <1.4 mg l^{-1})	1.513	1.145–1.999	0.004	1.429	1.009–2.023	0.044
Adjuvant therapy (yes *vs* no)	0.940	0.720–1.228	0.651	1.005	0.758–1.333	0.971

HR: hazard ratio; CI: confidential interval; LVI: lymph node invasion; CVH: concomitant variant histology; Cys-C: cystatin-C; eGFR: estimated glomerular filtration rate. -: not included in the analysis

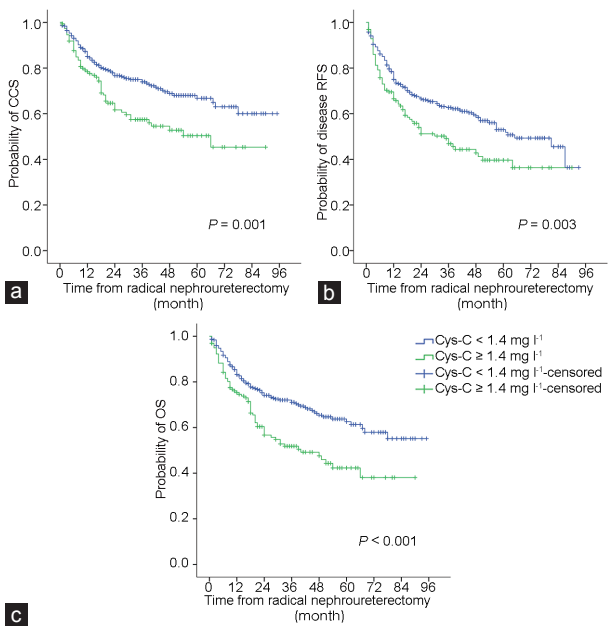

Figure 1: Kaplan–Meier curves and log-rank tests showing patients with higher preoperative serum Cys-C levels (Cys-C ≥1.4 mg l^{-1}, green) had worse (**a**) CSS ($P = 0.001$), (**b**) disease RFS ($P = 0.003$), and (**c**) OS ($P < 0.001$) compared with those with low Cys-C levels (Cys-C <1.4 mg l^{-1}, blue) in our cohort after radical nephroureterectomy. Cys-C: cystatin-C; CSS: cancer-specifc survival; RFS: recurrence-free survival; OS: overall survival.

of a recent meta-analysis which indicated that adjuvant chemotherapy was associated with OS and disease-free survival.[30]

Cys-C is an inhibitor of cysteine proteases which could facilitate the cell proliferation, differentiation, and migration *in vitro*.[31] Previous studies reported that serum Cys-C was elevated in patients with breast cancer, prostate cancer, colorectal cancer, melanoma, and ovarian cancer.[32–35] Recently, Yuan *et al*.[36] assessed 1063 nasopharyngeal carcinoma patients and they found that high serum Cys-C was independently associated with poor prognosis. Guo *et al*.[11] also found that high preoperative serum Cys-C was significantly associated with shorter OS and disease-free survival in 325 renal cell carcinoma patients. These findings were in line with the results of our cohort. However, the Cys-C was rarely detected or downregulated in various tumor tissues compared with that in normal tissues,[11,31,37,38] which indicated that Cys-C might be inhibited by tumorigenesis.

The researchers believed that the balance between Cys-C and cysteine proteases played an important role in tumor cell invasion and migration; however, the potential mechanisms remain unclear. Previous studies suggested that Cys-C could inhibit activities of cathepsins, a family of cysteine proteases that can promote cancer cell invasion and metastasis, and it was also involved in other activities related to tumor regression.[38] Cys-C was secreted into serum by immune cells and played a role in immune response to cancer-induced damage. Furthermore, cystatin family could also induce macrophages releasing nitric oxide and regulate cellular interleukin and cytokines in T-cells and fibroblasts and further modulate cell differentiation, proliferation,

Table 5: Univariable and multivariable Cox regression analyses of urinary tract urothelial carcinoma with regard to overall survival

Variable	*Univariable analysis*			*Multivariable analysis*		
	HR	*95% CI*	*P*	*HR*	*95% CI*	*P*
Age (per 1 year)	1.005	0.991–1.018	0.486	-	-	-
Sex (male *vs* female)	0.844	0.630–1.132	0.257	-	-	-
Tumor site			0.728			
Pelvicalyceal		Reference				
Ureteric	0.873	0.625–1.220	0.426	-	-	-
Both	0.958	0.638–1.437	0.835	-	-	-
Hydronephrosis (yes *vs* no)	1.255	0.921–1.709	0.150	-	-	-
Tumor grade (high *vs* low)	3.416	2.166–5.387	<0.001	1.914	1.147–3.195	0.013
Margin status (positive *vs* negative)	2.152	1.364–3.395	0.001	0.915	0.560–1.493	0.721
LVI (positive *vs* negative)	3.007	2.163–4.181	<0.001	1.365	0.933–1.996	0.109
CVH (with *vs* without)	2.414	1.769–3.294	<0.001	1.498	1.072–2.091	0.018
Tumor size (>3 cm *vs* ≤3 cm)	1.936	1.380–2.714	<0.001	1.449	1.011–2.076	0.044
Tumor architecture (sessile *vs* papillary)	3.023	2.042–4.476	<0.001	1.250	0.788–1.983	0.342
Surgical approach (laparoscopic *vs* open)	0.718	0.524–0.984	0.039	0.913	0.653–1.274	0.591
Perioperative blood transfusion (yes *vs* no)	2.064	1.432–2.974	<0.001	1.449	0.973–2.158	0.068
Tumor stage			<0.001			<0.001
pTa/is/1		Reference			Reference	
pT2 *vs* pTa/is/1	1.391	0.786–2.460	0.257	1.139	0.625–2.075	0.670
pT3 *vs* pTa/is/1	3.737	2.409–5.797	<0.001	2.554	1.534–4.250	<0.001
pT4 *vs* pTa/is/1	8.748	5.503–13.908	<0.001	4.047	2.176–7.528	<0.001
Lymph node status			<0.001			0.021
pN0		Reference			Reference	
pNx *vs* pN0	1.569	0.885–2.782	0.123	2.169	1.206–3.903	0.010
pN+ *vs* pN0	4.830	2.546–9.165	<0.001	2.479	1.259–4.883	0.009
eGFR (per 1 ml min^{-1} per 1.73 m^2)	0.994	0.987–1.001	0.095	1.010	1.001–1.019	0.024
Cys-C (≥1.4 mg l^{-1} *vs* <1.4 mg l^{-1})	1.791	1.327–2.416	<0.001	1.989	1.366–2.896	<0.001
Adjuvant therapy (yes *vs* no)	0.819	0.609–1.101	0.185	0.681	0.498–0.930	0.016

HR: hazard ratio; CI: confidential interval; LVI: lymph node invasion; CVH: concomitant variant histology; Cys-C: cystatin-C; eGFR: estimated glomerular filtration rate. -: not included in the analysis

and biological activities.[39,40] Therefore, high level of Cys-C may reflect the high level of inflammation and immune response *in vivo*, which may correspondingly indicate the high ability of malignancy and invasion of the tumors. However, how tumorigenesis conversely inhibits the expression of Cys-C in tumor tissues is still unknown. Furthermore, the expression level of Cys-C in UTUC tissues from patients' samples remains unclear either to date.

Some limitations in this study should be informed. First, the retrospective nature of this study may cause selection bias and cannot exclude potential confounding factors; in addition, lymphadenectomy is not routinely performed as there is no consensus on the lymphadenectomy pattern for UTUC and its additional benefits are still uncertain except for providing accurate staging and predicting survival.[1] The oncological outcomes could also be affected by surgical approaches, although there was no difference in surgical approaches between two groups. Indeed, this study is the first to explore the relationship of preoperative serum Cys-C and prognosis of UTUC. The results of the present study should be validated by future prospective studies, and the potential mechanisms should be further investigated.

CONCLUSIONS

The preoperative serum Cys-C level was not only a predictor of renal function but also proved to be an independent prognostic predictor in patients undergoing RNU for UTUC. Cys-C may act as a useful biomarker to preoperatively select high-risk patients who may need adjuvant therapy and should be monitored more frequently after surgery.

AUTHOR CONTRIBUTIONS

PT and MS participated in project design and performed data collection and statistical analysis. PT drafted the manuscript. MS did the paper revision. JC, NX, and Hang X participated in data collection and manuscript revision. Huan X and YJ reviewed all specimens and made pathology classification. JZA and LRL participated in data collection and helped to analyze data. LY and QW carried out project design and participated in data explanation and manuscript revision. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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